Biological aspects of deciphering and engineering regulatory networks

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In efforts to detect and modify regulation of pathways for a particular goal there are strategies that can be implemented in two circumstances, one the synthetic approach where major components of the pathway are known and the appropriate regulation of the various enzymes can be adjusted by taking advantage of modeling and combinatorial assembly methods; and the other if the proteins to be altered are not obvious and thus the modification must take a more empirical approach with selection or screening methods being the issues.

In known expression systems, it is still needed to detect levels of regulation, for example determination of the level of functioning of a protein (enzyme for metabolic process) more than knowledge of the level of gene expression is needed to understand how the activity varies under the physiological condition contemplated for use. This is the situation if genes from various sources are placed together in a new way to form a non-endogenous pathway as often is the case in metabolic engineering. Detection of proteins, metabolites etc from systems biology approach of measurements in different genetically engineered cells under various conditions can help in this endeavor. Due to the large number of possible combinations of mutations and conditions a way is needed to minimize or focus the experimental measurements on the most appropriate ones to examine. Such experiments should make an effort to take into account the effects of intercellular conditions produced by introduced changes on related protein activities (other enzymes of pathway, regulatory factors, functional state of activity of the enzyme or regulatory proteins) and models that include this interaction information would be more comprehensive.

In order to carry out appropriate modification of regulation there are relatively straightforward approaches in the case of desired changes in specific known expression controls such as through modification of transcriptional events and to a lesser extent, modification of more general aspects of cellular physiology (redox and air, enzyme stability, enzyme parameters, osmotic conditions). To target regulation to specific pathway, designed regulation of small units can be employed. These involve the use of known regulated promoters that are varied to adjust the level of constitutive expression, and can be combined with terminators or RNA structural elements to afford variation of level of expression. In the case of less known processes found in many industrial organisms that have less genetic and biochemical literature, efforts are more a matter of perturbing a somewhat more global functioning system and screening or selection for those altered cells that perform better, then analyzing and combining the most promising. The idea of eliminating complicating or undesired processes that may obscure the regulation you would like to enhance is an useful experimental strategy. For these wider scope effects, or regulation of unknown factors with less obvious connections to the metabolically engineered process, modification of transcription factors such as sigma factors, Zn-finger motif factors, general or global transcription factors may be used in combination with powerful selection or screening systems for the desired property.